510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE ONLY TEMPLATE

A. 510(k) Number:
K032897

B. Analyte:
Clostridium difficile Toxin A & B

C. Type of Test:
Enzyme immunoassay

D. Applicant:
IVD Research Inc.

E. Proprietary and Established Names:
Clostridium difficile toxin A+B Fecal Antigen Detection Microwell ELISA

F. Regulatory Information:

1. Regulation section:
   21 CFR Part 866.2660 Microorganism Differentiation and Identification Device
2. Classification:
   Class I
3. Product Code:
   LLH – Reagents, Clostridium difficile toxin
4. Panel:
   83 (Microbiology)

G. Intended Use:

1. Intended use(s):
   This microwell enzyme linked immunoabsorbant assay (ELISA) detection kit is an *in vitro* diagnostic (IVD) immunoassay for the detection of antigen to *C. difficile* A and B toxins in human feces using peroxidase as the indicator enzyme. The assay may be read visually or with and ELISA reader. This ELISA Kit is intended to be used with stools that are fresh, frozen or in Cary-Blair transport media. The assay is intended for use as an aid in diagnosis of *C. difficile* associated disease.
2. Indication(s) for use:
   This microwell enzyme-linked immunoabsorbant assay (ELISA) detection kit (C. difficile Toxin A+B ELISA Kit) is an in vitro diagnostic (IVD) immunoassay intended for use as an aid in the diagnosis of *C. difficile* associated disease. The kit detects *C. difficile* toxin A and B in human feces using peroxidase as the indicator enzyme. The assay may be read visually or with an ELISA reader. This IVD C. difficile Toxin A+B ELISA Kit is intended to be used with human stools that are fresh, frozen or in Cary Blair transport media in a clinical laboratory use setting. The kit may also be used with IVD Research's Quick'N'Easy fecal dilution device.
3. Special condition for use statement(s):
   Prescription Use

4. Special instrument Requirements:
   Not applicable

H. Device Description:

The kit consists of 96 test wells coated with rabbit anti-*C. difficile* Toxin A and B; conjugate consisting of peroxidase labeled chicken anti-Toxin A and B; positive and negative controls; sample diluent; wash buffer; color substrate, stop solution; transfer pipettes, procedure card; instructions for use and a plate cover.

I. Substantial Equivalence Information:

1. Predicate device name(s):
   Meridian Premier Toxins A&B
   Biostar *C. difficile* TOX A OIA
2. Predicate K number(s):
   K926442
   K991829
3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate (s)**
Intended Use	Detection of *C. difficile* Toxins A and B in fecal specimens	Detection of *C. difficile* Toxins A and B in fecal specimens
Technology	Enzyme immunoassay	Enzyme immunoassay
Material : device	Microwell	Microwell
Material: conjugate	Horseradish peroxidase conjugated to anti-toxins	Horse radish peroxidase conjugated to anti-toxins
Specimen type	Fresh human stool specimens or specimens in modified Cary-Blair	Fresh human stool specimens
Differences		
Item	**Device**	**Predicate (s)**
Capture antibodies or molecules:device	chicken polyclonal anti-Toxin A+B and rabbit polyclonal anti-Toxin A +B	Biostar: rabbit antibody against Toxin A Meridian: Mouse monoclonal anti-Toxin A and polyclonal goat anti-Toxin B
Antibodies: conjugate	Chicken polyclonal anti-Toxin A and B	Biostar: rabbit Toxin A antibody Meridian: Polyclonal goat anti-Toxin A and anti-Toxin B
Sample volume	100µl	same

J. Standard/Guidance Document Referenced (if applicable):

CDRH Guidance Document for Industry and FDA Staff: "Review Criteria for assessment of laboratory tests directed at assisting in the diagnosis of *C.difficile* associated disease"

K. Test Principle:

The *Clostridium difficile* toxin A+B Fecal Antigen Detection Microwell ELISA test detects the presence of Toxin A and Toxin B in clinical stool specimens. The microwells are coated with rabbit polyclonal anti-Toxins A+B. A stool specimen is diluted in Sample Diluent or used directly if pre-diluted in modified Cary-Blair medium. The sample is added to a microwell allowing the toxins, if present, to bind to the immobilized antibodies. After washing to remove unbound components, a conjugate reagent containing anti-chicken polyclonal antibodies conjugated to peroxidase is added to each well. Unbound conjugate is removed by washing and a chromogenic substrate, tetramethylbenzidine (TMB) solution, is added to detect the presence of bound toxin. A stop reagent is added and the test results are read visually or spectrophotometrically. The presence of a yellow color indicates the presence of antigen to *C. difficile* A + B toxins.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Reproducibility testing was conducted at three sites on three consecutive days with eleven blinded samples. The specimens included two negative specimens and nine positive specimens with varying levels of reactivity. The average inter-assay coefficient of variation (CV) range for the negative samples was .49-.62. The average inter-assay CV range for the positive samples was 0.2-.17.

b. Linearity/assay reportable range:
Not applicable

c. Traceability (controls, calibrators, or method):
Not applicable

d. Detection limit:
The *Clostridium difficile* toxin A+B Fecal Antigen Detection Microwell ELISA test kit detects Toxin A at levels of $\geq$2.0 ng/ml and Toxin B at levels of $\geq$3.0 ng/ml.

e. Analytical specificity:

Thirty (30) microorganisms were evaluated with the *Clostridium difficile* toxin A+B Fecal Antigen Detection assay. Bacteria isolates were tested at $\geq 10^8$ colony-forming units per ml (cfu/ml). No cross-reactivity was observed with all isolates except *Clostridium sordellii*. The following organisms were tested in the *Clostridium difficile* Toxin A+B Microwell assay.

Organism
Bacteriodes fragilis
Campylobacter coli
Campylobacter jejuni
Campylobacter fetus
Candida albicans

Organism
Clostridium haemolyticum
Clostridium perfringens
Clostridium septicum
Clostridium sordelleii
Clostridium sporogenes
Clostridium novyi
Citrobacter braakii
Enterobacter cloacae
Enterococcus faecalis
Escherichia coli
Escherichia hermanii
Helicobacter cinaedi
Klebsiella pneumoniae
Proteus vulgaris
Pseudomonas aeruginosa
Salmonella choleraesuis (typhimurium)
Salmonella hadar
Salmonella infantis
Salmonella enteriditis
Serratia liquefaciens
Shigella dysenteriae
Shigella flexneri
Shigella sonnei
Staphylococcus aureus
Yersinia enterocolitica

f. Assay cut-off:
The assay was determined to detect Toxin A at levels of ≥ 2.0ng/ml and Toxin B at ≥ 3.0 ng/ml. Concentrations of purified toxins were assigned from serial dilution results obtained through either gold standard testing or by testing in a predicate device. Concentration of toxin testing close to the assay cut off was determined. The toxins were also titrated beyond the assay cut off on the test device. The last dilution remaining at or above the assay cut off was defined as the endpoint dilution. The highest negative and the lowest positive sample toxin concentrations were plotted versus OD values were used to calculate the concentration at the OD cut off assay value.

2. Comparison studies:
   a. *Method comparison with predicate device:*
      See below studies 3, 4 (Part B), and 5.
   b. *Matrix comparison:*
      Not applicable
3. Clinical studies:
   a. *Clinical sensitivity:*

Sensitivity/Specificity

Study #1 (Toxin B Cell Culture)

A total of 69 stools were tested against a cytotoxin B cell culture procedure. The following results were obtained.

	Cyto B +	Cyto B -
IVD ELISA +	14	2
IVD ELISA -	4	49

Sensitivity: 78% (14/18) 95% CI = 52% to 94%
Specificity: 96% (49/51) 95% CI = 87% to 100%

Study #2 (In House Study)

A total of 24 stools in Cary Blair transport media (5 positive, 19 negative) were tested in this ELISA. In addition, 14 fresh/frozen stools (6 positive, 8 negative) were diluted using the Quick'N'Easy Fecal Sample Prep device and tested in the ELISA. The following results were obtained.

	ELISA/OIA +	ELISA/OIA -
IVD ELISA +	11	0
IVD ELISA -	0	27

Positive Agreement = 100% (11/11)
Negative Agreement = 100% (27/27)

Study #3 (Mid-West Clinical Lab)

This study compared 53 fresh samples versus another commercial A+B ELISA. The IVD ELISA had a positive agreement of 100% (2/2) and a negative agreement of 98% (50/51).

Study #4 (Reference #17)

A total of 311 stools were tested against culture and another commercial toxin A+B ELISA kit.
The following results were obtained.

	Culture +	Culture –
IVD ELISA +	49	8
IVD ELISA -	33	221

Sensitivity: 60% (49/82) 95% CI = 48% to 70%
Specificity: 97% (221/229) 95% CI = 93% to 99%

The other commercial ELISA showed a sensitivity of 66% (54/82) and a specificity of 98% (225/229) on the same set of samples.
Between the two ELISA's there was a positive agreement of 91% (49/54) and a negative agreement of 98% (221/225).

Study #5 (East Coast Hospital Lab)

This study compared 82 fresh or frozen samples versus another commercial Toxin A Only Optical Immunoassay (OIA). The IVD ELISA had a positive agreement of 79% (27/34) and a negative agreement of 94% (45/48).

b. Clinical specificity:
Refer to (a) above
c. Other clinical supportive data (when a and b are not applicable):
Not applicable

4. Clinical cut-off:
   See assay cut-off above
5. Expected values/Reference range:

A positive reaction indicates that the patient is shedding detectable amounts of *C. difficile* antigen. The frequency of *C. difficile* disease is dependent on various factors such as the type of institution, patient population and potential outbreak status. Asymptomatic carrier rates have been reported from a low of 2% in Sweden to a high of 15% in Japan. Hospitalized patients taking certain antibiotics are at high risk of acquiring C. difficile with infection rates of 21% being reported in one study. A recent article in Journal of Clinical Microbiology (ref. #18) provides a good overview of testing for *C. difficile*. Further information on *C. difficile* and antibiotic colitis can also be found in the Manual of Clinical Microbiology, ASM Press, 7th Edition.

M. Conclusion:
The submitted information in this premarket notification is complete and supports a substantial equivalence decision.